Soft X-Ray Microscopy of Parasitic Protozoa

W. J. Kozek[1], S. Garlapati[2], C.C. Wang[2], A. Habteab[3],
G. Denbeaux[3], A. Pearsons[3] and C. A. Larabell[4]

[1]Department of Microbiology and Medical Zoology, Medical Sciences Campus,
University of Puerto Rico, San Juan, PR
[2]Department of Chemistry, School of Pharmacy, University of California,
San Francisco, CA
[3]Divisions of X-Ray Optics and [4]Life Sciences, Advanced Light Source,
E.O. Lawrence Berkeley National Laboratory, Berkeley, CA

One of the most common intestinal protozoan parasites of man in the United States is *Giardia lamblia*. Children are more frequently affected than adults, although all infected persons can have symptoms ranging from mild diarrhea, flatulence, crampy abdominal pains, anorexia and epigastric tenderness to steatorrhea and full-blown malabsorption syndrome. The life cycle of this parasite consists of two stages: the cyst – the infective stage, and the trophozoite, the vegetative stage that inhabits upper small intestine. This infection is principally acquired by ingestions of cysts in water, less commonly as contaminants of ingested food. In the United States it is considered to be the main cause of diarrheal outbreaks from contaminated water supplies. Geographic strains of varying pathogenicity lead to inclusion of giardiasis in the differential diagnosis of "traveler's diarrhea" in patients returning from abroad. Beavers, muskrats and voles serve as reservoir hosts which can transmit this infection to campers, backpackers and mountain climbers; cattle, sheep and dogs may be serve as reservoir hosts of infection in paradomestic settings. Day care centers can be sites of significant endemic giardiasis and transmission.

Since our previous investigations have demonstrated the usefulness of soft X-ray microscopy to examine small, multicellular organisms, we have extended these studies to determine whether relatively fragile protozoa can also be effectively examined by soft X-ray microscopy. As the subject we have used trophozoites of *G. lamblia*. This relatively fragile trophozoite, 9 – 20μ by 5 – 15μ in size, can be cultured *in vitro,* and can be recovered in large numbers, for a variety of biomedical investigations. Culture-grown trophozoites were fixed in cold 3.5% glutaraldehyde for three to four hours, washed twice in Millonig's buffer pH 7.3, 30 mins/ wash, then rinsed three times in distilled water, 15 mins/rinse. Suspensions of fixed trophozoites in water were examined in the XM-1 soft X-ray microscope.

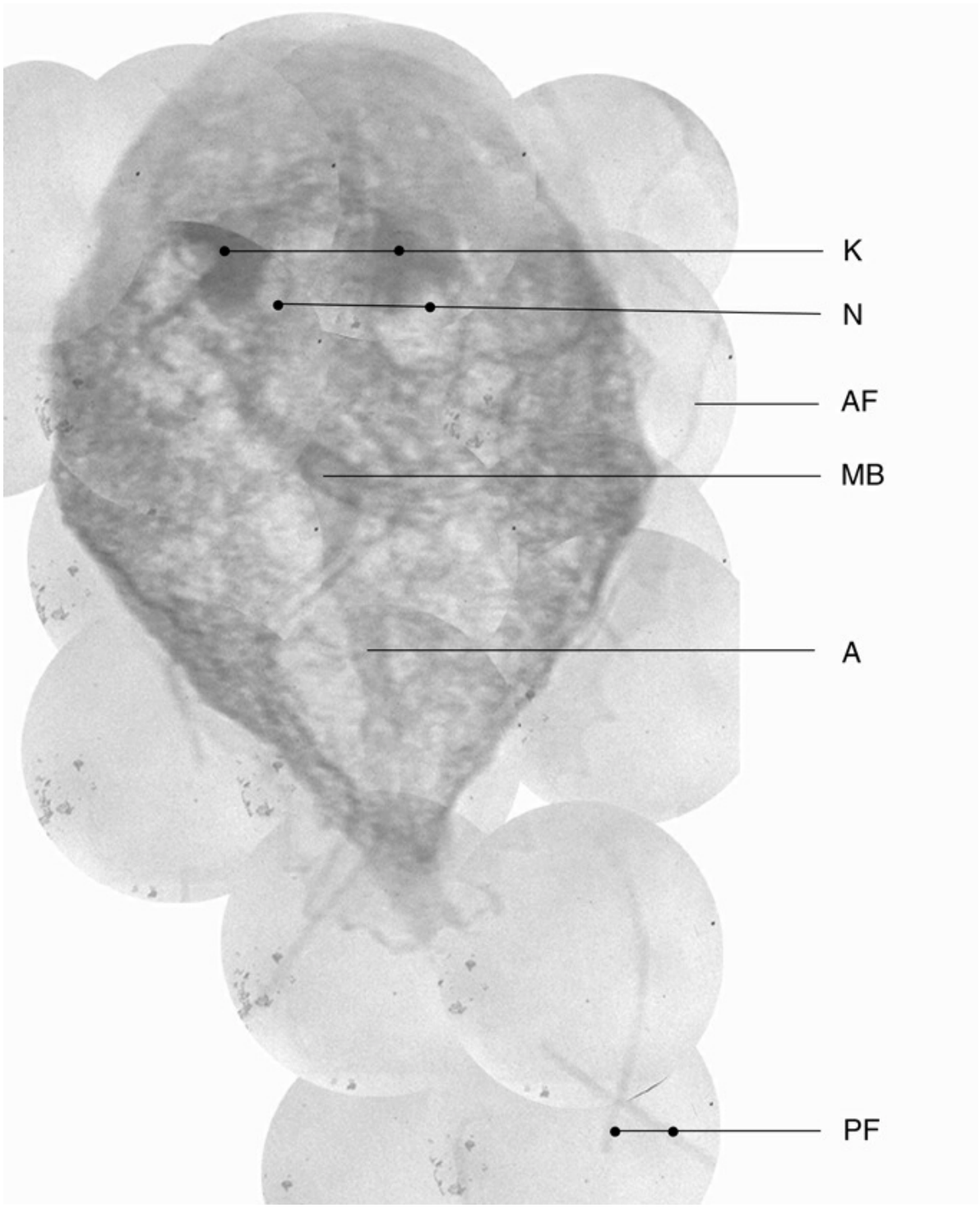

Figure 1. Tiled image of a trophozoite of *Giardia lambila*, dorsal aspect. K-karyosome (nucleolus) in each of the two nuclei (N), AF – anterior flagellum, MB – medial bodies, A – axonemes, PF – posterior flagellum.

Numerous trophozoites curled laterally during fixation, which rendered them less useful for more detailed examination. However, many other trophozoites were fixed in resting position suitable for closer observation. Figure 1demonstrates that the fixed trophozoite is sufficiently sturdy to withstand significant time period in the beam to allow sequential exposures to be taken for the preparation of a montage or a tile. The leucent cytoplasm of the trophozoite contains many clear vacuoles. The following internal structures are readily discernible: the two nuclei (N), each with its prominent nucleolus (K), and median bodies (MB) located almost at right angle to the axostyles (A) that originate in the central area anterior to the nuclei. The extension of the eight flagella varied from trophozoite to trophozoite; posterior flagella were more readily discernible that the anterior. The dorsal orientation of the trophozoite prevented the visualization of the adhesive disc, located on the ventral aspect, which is used by *Giardia* for adhesion to the surface of mucosal cells.

These preliminary trials have demonstrated that the relatively fragile protozoa can also be effectively examined by soft X-ray microscopy, despite the lack of the more rigid cuticle that envelops the nematode larvae. These studies will be continued to elucidate the complete structure of the trophozoite, and will be expanded to include the examination of other organisms of public health significance.

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Principal Investigator: Wieslaw J. Kozek, Department of Microbiology and Medical Zoology, University of Puerto Rico. E-mail address: wkozek@rcm.upr.edu. Telephone: 787-759-8257.